Reviewer's report

Title: Qualitative and Quantitative Proteomic Analysis of Nipple Aspirate Fluid from Women with Early-Stage Breast Cancer using Isotope-coded Affinity Tags and Tandem Mass Spectrometry

Version: 3 Date: 27 February 2006

Reviewer: Emanuel Petricoin

Reviewer's report:

Summary:
The authors have addressed the issues that Reviewers 1 and 2 have raised. While the reciprocal ICAT experiment as suggested by Reviewer 3 would have been nice to see, it is impossible to perform this experiment now as the samples have been exhausted by the previous analysis. Of the entirety of protein candidates identified as being significantly altered in the matched sample set, only the Vit D binding protein was Western confirmed.

Comment:
1. I feel that Reviewer 3 is correct in his assertion that the title is a bit deceiving in the depth the paper attains insofar as generating more global confident results. I would recommend a title that tries to split the difference a bit. Something like: "Proteomic Analysis of Nipple Aspirate Fluid from Women with Early-Stage Breast Cancer using Isotope-coded Affinity Tags and Tandem Mass Spectrometry Reveals Differential Expression of Vitamin D Binding Protein".

2. The use of patient matched affected and unaffected NAF is a very very powerful study set-ignored by the reviewers in the clarity it can provide to differentially expressed proteins and the potential problem of potential sample bias. Coupling the well controlled study set used for discovery to the fact that the reviewers did confirm one of the proteins by western, and the fact that the authors did not appear to be making global conclusions and appeared circumspect in the discussion, I feel that the paper should be accepted. Furthermore, I feel that ROC curve analysis from western blots may not be the best approach since westerns are, in this iteration, inherently a non-quantitative analysis and do not provide accurate cut-points. The authors do not appear to be making over-optimistic statements about the clinical accuracy of the Vit D binding protein and state that more experimentation is needed.

Declaration of competing interests:

I am an inventor on US Government assigned and University held issued patents and patents pending that cover broad areas of proteomic technologies and specific biomarkers for a variety of cancers including breast cancer. I am entitled to receive royalties on licenses taken on these patents.